OR OFFICIAL USE ONLY 160328 ACCESS DB # _____ PLEASE PRINT CLEARLY

Scientific and Technical Information Center CRFE

SEARCH REQUEST FORM

equester's Full Name: DAVID GUZO Examiner # : 70677 Date: 7/25/05
rt Unit: 1636 Phone Number: 2-0767 Serial Number: 10/738454
ocation (Bldg/Room#): Remsen 2A-15 (Mailbox #): 2C70 Results Format Preferred (circle): PAPER DISK

****

) ensure an efficient and quality search, please attach a copy of the cover sheet, claims, and abstract or fill out the following:

itle of Invention: _____

ıventors (please provide full names): _____

_____

arliest Priority Date: _____

earch Topic:
lease provide a detailed statement of the search topic, and describe as specifically as possible the subject matter to be searched. Include the ected species or structures, keywords, synonyms, acronyms, and registry numbers, and combine with the concept or utility of the invention. efine any terms that may have a special meaning. Give examples or relevant citations, authors, etc., if known.

For Sequence Searches Only* *Please include all pertinent information (parent, child, divisional, or issued patent numbers) along with the ppropriate serial number.*

Please run a regular plus interference sequence search on SEQ ID NO: 24.

1

147 NA

Thanks

****

STAFF USE ONLY

Searcher: _____
Searcher Phone #: _____
Searcher Location: _____
Date Searcher Picked Up: 7/27/05
Date Completed: 8/1/05
Searcher Prep & Review Time: _____
Online Time: _____

Type of Search

__/__ NA Sequence (#)
_____ AA Sequence (#)
_____ Structure (#)
_____ Bibliographic
_____ Litigation
_____ Fulltext
_____ Other

Vendors and cost where applicable

_____ STN _____ Dialog
_____ Questel/Orbit _____ Lexis/Nexis
_____ Westlaw _____ WWW/Internet
__/__ In-house sequence systems
__ Commercial __ Oligomer __ Score/Length
__ Interference __ SPDI __ Encode/Transl
_____ Other (specify)

GenCore version 5.1.6

OM nucleic - nucleic search, using sw model

Run on: July 29, 2005, 09:50:01 ; Search time 3214 Seconds
(without alignments)
8846.923 Million cell updates/sec

Title: US-10-738-454-24
Perfect score: 747
Sequence: 1 gacgtcgcagtcacccaaag.....ttgttctaccatacatctag 747

Scoring table: IDENTITY_NUC
Gapop 10.0 , Gapext 1.0

Searched: 34239544 seqs, 19032134700 residues

Total number of hits satisfying chosen parameters: 68479088

Minimum DB seq length: 0
Maximum DB seq length: 2000000000

Post-processing: Minimum Match 0%
Maximum Match 100%
Listing first 45 summaries

Database : EST:*
1: gb_est1:*
2: gb_est2:*
3: gb_htc:*
4: gb_est3:*
5: gb_est4:*
6: gb_est5:*
7: gb_est6:*
8: gb_gss1:*
9: gb_gss2:*

Pred. No. is the number of results predicted by chance to have a score greater than or equal to the score of the result being printed, and is derived by analysis of the total score distribution.

SUMMARIES

	Result No.	Score	% Query Match	Length	DB	ID	Description
	1	278.2	37.2	344	9	AY403874	AY403874 Mus muscu
	2	277.4	37.1	420	5	BQ562821	BQ562821 H4078B09-
	3	277.4	37.1	450	6	CA574865	CA574865 K0622C03-
	4	277.4	37.1	576	6	CA574702	CA574702 K0619H08-
c	5	274.4	36.7	856	9	BX992686	BX992686 Forward s
	6	273.8	36.7	3115	3	AK088744	AK088744 Mus muscu
	7	268	35.9	623	2	BB637181	BB637181 BB637181
	8	268	35.9	626	2	BB631546	BB631546 BB631546
	9	268	35.9	653	2	BB636280	BB636280 BB636280
	10	268	35.9	664	2	BB638804	BB638804 BB638804
	11	268	35.9	1507	3	AK040993	AK040993 Mus muscu
	12	268	35.9	1638	3	AK080320	AK080320 Mus muscu
	13	268	35.9	3978	3	AK038154	AK038154 Mus muscu
	14	268	35.9	4009	3	AK040590	AK040590 Mus muscu
	15	267.4	35.8	633	2	BB654289	BB654289 BB654289
	16	267.4	35.8	678	6	BY746975	BY746975 BY746975
c	17	265.6	35.6	636	6	BY717844	BY717844 BY717844
	18	253	33.9	844	7	CO809315	CO809315 AGENCOURT
	19	244.8	32.8	828	7	CO566088	CO566088 AGENCOURT
	20	231.4	31.0	756	7	CO560706	CO560706 AGENCOURT
	21	227.6	30.5	352	1	AI182165	AI182165 uc64c08.r
	22	226	30.3	745	6	CB598341	CB598341 AGENCOURT
	23	226	30.3	754	7	CO559036	CO559036 AGENCOURT
	24	224.6	30.1	786	7	CO556346	CO556346 AGENCOURT
	25	223.6	29.9	635	7	CF910965	CF910965 A0608A06-
	26	215.8	28.9	853	7	CO571935	CO571935 AGENCOURT
	27	212.2	28.4	452	8	BZ154671	BZ154671 CH230-347
	28	208.8	28.0	359	5	BY058200	BY058200 BY058200
c	29	197.2	26.4	776	8	BZ225992	BZ225992 CH230-352
	30	197	26.4	813	4	BI906836	BI906836 603064619
	31	195.6	26.2	557	9	CR192991	CR192991 Forward s
c	32	194	26.0	835	8	BZ105577	BZ105577 CH230-239
	33	188	25.2	624	6	CD703346	CD703346 EST19873
c	34	186.6	25.0	848	8	BZ094248	BZ094248 CH230-141
	35	185	24.8	211	7	CF911672	CF911672 A0619B08-
	36	184.8	24.7	418	5	BY224348	BY224348 BY224348
	37	177.8	23.8	344	9	AY403872	AY403872 Homo sapi
	38	177.2	23.7	776	6	CB957360	CB957360 AGENCOURT
	39	175.4	23.5	807	5	BP163924	BP163924 BP163924
	40	175.2	23.5	392	5	BX456695	BX456695 BX456695
	41	174.8	23.4	709	5	BP163546	BP163546 BP163546
	42	174.6	23.4	540	1	AA874473	AA874473 vx80g02.r
	43	170.4	22.8	814	5	BP169000	BP169000 BP169000
	44	167.2	22.4	334	9	AY403873	AY403873 Pan trogl
	45	162.4	21.7	756	4	BG540421	BG540421 602568733

ALIGNMENTS

RESULT 1
AY403874
LOCUS AY403874 344 bp DNA linear GSS 12-DEC-2003
DEFINITION Mus musculus HCM1702 gene, VIRTUAL TRANSCRIPT, partial sequence, genomic survey sequence.
ACCESSION AY403874
VERSION AY403874.1 GI:39759857
KEYWORDS GSS.
SOURCE Mus musculus (house mouse)
ORGANISM Mus musculus
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Rodentia; Sciurognathi; Muridae; Murinae; Mus.
REFERENCE 1 (bases 1 to 344)
AUTHORS Clark,A.G., Glanowski,S., Nielson,R., Thomas,P., Kejariwal,A., Todd,M.A., Tanenbaum,D.M., Civello,D.R., Lu,F., Murphy,B., Ferriera,S., Wang,G., Zheng,X.H., White,T.J., Sninsky,J.J., Adams,M.D. and Cargill,M.
TITLE Inferring nonneutral evolution from human-chimp-mouse orthologous gene trios
JOURNAL Science 302 (5652), 1960-1963 (2003)
PUBMED 14671302
REFERENCE 2 (bases 1 to 344)
AUTHORS Clark,A.G., Glanowski,S., Nielson,R., Thomas,P., Kejariwal,A., Todd,M.A., Tanenbaum,D.M., Civello,D.R., Lu,F., Murphy,B., Ferriera,S., Wang,G., Zheng,X.H., White,T.J., Sninsky,J.J., Adams,M.D. and Cargill,M.
TITLE Direct Submission
JOURNAL Submitted (16-NOV-2003) Celera Genomics, 45 West Gude Drive, Rockville, MD 20850, USA
COMMENT This sequence was made by sequencing genomic exons and ordering them based on alignment.
FEATURES Location/Qualifiers
source 1. .344
/organism="Mus musculus"
/mol_type="genomic DNA"
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ORIGIN

Query Match 37.2%; Score 278.2; DB 9; Length 344;
Best Local Similarity 98.9%; Pred. No. 7.2e-73;
Matches 280; Conservative 0; Mismatches 3; Indels 0; Gaps 0;

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Qy          1 GACGTCGCAGTCACCCAAAGCCCAAGAAACAAGGTGGCAGTAACAGGAGGAAAGGTGACA 60
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Db         58 GAGGCTGCAGTCACCCAAAGCCCAAGAAACAAGGTGGCAGTAACAGGAGGAAAGGTGACA 117
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GenCore version 5.1.6

OM nucleic - nucleic search, using sw model

Run on: July 29, 2005, 03:19:14 ; Search time 533 Seconds
(without alignments)
8296.523 Million cell updates/sec

Title: US-10-738-454-24
Perfect score: 747
Sequence: 1 gacgtcgcagtcacccaaag.....ttgttctaccatacatctag 747

Scoring table: IDENTITY_NUC
Gapop 10.0 , Gapext 1.0

Searched: 4390206 seqs, 2959870667 residues

Total number of hits satisfying chosen parameters: 8780412

Minimum DB seq length: 0
Maximum DB seq length: 2000000000

Post-processing: Minimum Match 0%
Maximum Match 100%
Listing first 45 summaries

Database : N_Geneseq_16Dec04:*
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3: geneseqn2000s:*
4: geneseqn2001as:*
5: geneseqn2001bs:*
6: geneseqn2002as:*
7: geneseqn2002bs:*
8: geneseqn2003as:*
9: geneseqn2003bs:*
10: geneseqn2003cs:*
11: geneseqn2003ds:*
12: geneseqn2004as:*
13: geneseqn2004bs:*

Pred. No. is the number of results predicted by chance to have a score greater than or equal to the score of the result being printed, and is derived by analysis of the total score distribution.

SUMMARIES

Result No.	Score	% Query Match	Length	DB	ID	Description
1	747	100.0	747	2	AAX87213	Aax87213 Yeast sur
2	747	100.0	747	2	AAD27168	Aad27168 Cell surf
3	747	100.0	747	4	AAD21543	Aad21543 Single ch
4	747	100.0	747	6	ABS53657	Abs53657 T-cell re
5	747	100.0	747	12	ADJ88104	Adj88104 Surface T
6	745.4	99.8	747	13	ADR03286	Adr03286 DNA encod
7	342.2	45.8	1372	1	AAN60166	Aan60166 Sequence
8	281.2	37.6	1350	2	AAT97844	Aat97844 DNA of a
9	278.8	37.3	471	2	AAT31599	Aat31599 Beta chai
10	278.8	37.3	954	2	AAX25358	Aax25358 Mouse A1
11	278.6	37.3	366	2	AAQ50973	Aaq50973 Vbeta TCR
12	278.6	37.3	366	5	AAF24745	Aaf24745 Nucleotid
13	278.6	37.3	366	6	ABN84207	Abn84207 T cell re
14	278.6	37.3	366	10	ADJ33890	Adj33890 Mouse DNA
15	278.6	37.3	747	5	AAF24747	Aaf24747 Nucleotid
16	278.6	37.3	747	6	ABN84209	Abn84209 T cell re
17	278.6	37.3	747	10	ADJ33894	Adj33894 Mouse DNA
18	278.6	37.3	753	2	AAQ50974	Aaq50974 scValphaV
19	278.6	37.3	753	5	AAF24746	Aaf24746 Nucleotid
20	278.6	37.3	753	6	ABN84208	Abn84208 T cell re
21	278.6	37.3	753	10	ADJ33892	Adj33892 Mouse DNA
22	277.8	37.2	796	2	AAT29757	Aat29757 D10 singl
23	277	37.1	747	2	AAQ50975	Aaq50975 scValphaV
24	274.2	36.7	303	2	AAT87038	Aat87038 T cell re
25	266.4	35.7	1131	12	ADL27458	Adl27458 Nucleotid
26	243.6	32.6	912	2	AAX36391	Aax36391 Killer T-
27	238.8	32.0	916	6	AAD42573	Aad42573 Murine TC
28	236.6	31.7	402	2	AAT97842	Aat97842 Beta chai
29	225.2	30.1	409	10	ABT41755	Abt41755 Toxicity
30	221.2	29.6	290	2	AAQ32717	Aaq32717 TCR V-bet
31	195.4	26.2	581	12	ACH68874	Ach68874 Human gen
32	188	25.2	569	2	AAQ28125	Aaq28125 Human T l
33	177.8	23.8	1080	13	ACN41343	Acn41343 Human dia
34	177.2	23.7	733	8	ACD05953	Acd05953 Novel hum
35	176.4	23.6	291	12	ACH82574	Ach82574 Human gen
36	176.2	23.6	654	8	ACD05711	Acd05711 cDNA enco
37	176.2	23.6	1100	12	ADH70071	Adh70071 Human Vbe
38	176.2	23.6	1246	12	ADH70073	Adh70073 Human Vbe
39	176.2	23.6	110000	12	ADH69807_2	Continuation (3 of
40	176.2	23.6	110000	12	ADH69807_3	Continuation (4 of
41	176.2	23.6	267156	6	ABL68560	Abl68560 Kidney ca
42	171.6	23.0	294	2	AAQ28183	Aaq28183 Human T l
43	171.4	22.9	526	8	ABX63789	Abx63789 Human cDN
44	169.8	22.7	1207	12	ADH70072	Adh70072 Human Vbe
c 45	164.4	22.0	402	3	AAZ91133	Aaz91133 Canine T-

ALIGNMENTS

RESULT 1
AAX87213
ID AAX87213 standard; DNA; 747 BP.
XX
AC AAX87213;
XX
DT 27-SEP-1999 (first entry)
XX
DE Yeast surface displayed T cell receptor.
XX
KW T cell receptor; monoclonal antibody KJ16; scFv; agglutinin; yeast;
KW antibody engineering; surface display; protein library; peptide library;
KW cancer; sepsis; autoimmune disease; arthritis; diabetes;
KW multiple scelerosis; therapy; ss.
XX
OS Mammalia.
OS Synthetic.
XX
FH Key Location/Qualifiers
FT mutation replace(50,A)
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FT /note= "G17E"
FT mutation replace(533,C)
FT /*tag= b
FT /note= "L43P"
FT mutation replace(701,C)
FT /*tag= c
FT /note= "L104P"
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PN WO9936569-A1.
XX
PD 22-JUL-1999.
XX
PF 20-JAN-1999; 99WO-US001188.
XX
PR 20-JAN-1998; 98US-00009388.
PR 26-AUG-1998; 98US-00140084.
XX
PA (UNII) UNIV ILLINOIS FOUND.
XX
PI Wittrup KD, Kieke MC, Kranz DM, Shusta E, Boder ET;
XX
DR WPI; 1999-430619/36.

GenCore version 5.1.6

OM nucleic - nucleic search, using sw model

Run on: July 29, 2005, 06:57:21 ; Search time 3600 Seconds
(without alignments)
10054.457 Million cell updates/sec

Title: US-10-738-454-24
Perfect score: 747
Sequence: 1 gacgtcgcagtcacccaaag.....ttgttctaccatacatctag 747

Scoring table: IDENTITY_NUC
Gapop 10.0 , Gapext 1.0

Searched: 4708233 seqs, 24227607955 residues

Total number of hits satisfying chosen parameters: 9416466

Minimum DB seq length: 0
Maximum DB seq length: 2000000000

Post-processing: Minimum Match 0%
Maximum Match 100%
Listing first 45 summaries

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2: gb_htg:*
3: gb_in:*
4: gb_om:*
5: gb_ov:*
6: gb_pat:*
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8: gb_pl:*
9: gb_pr:*
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11: gb_sts:*
12: gb_sy:*
13: gb_un:*
14: gb_vi:*

Pred. No. is the number of results predicted by chance to have a score greater than or equal to the score of the result being printed, and is derived by analysis of the total score distribution.

SUMMARIES

Result No.	Score	% Query Match	Length	DB	ID	Description
1	747	100.0	747	6	AR220110	AR220110 Sequence
2	747	100.0	747	6	AR369282	AR369282 Sequence
3	747	100.0	747	6	AR476748	AR476748 Sequence
4	747	100.0	747	6	AR478145	AR478145 Sequence
5	342.2	45.8	1372	6	E01014	E01014 cDNA encodi
6	342.2	45.8	1372	6	I02550	I02550 Sequence 1
7	342.2	45.8	1372	6	AR362954	AR362954 Sequence
8	342.2	45.8	1372	10	MMTCEL11	X01134 M.musculus
9	294.8	39.5	435	10	MMTCRA2	X05736 Mouse mRNA
10	283	37.9	852	12	SCU59428	U59428 Synthetic c
11	281	37.6	2625	6	AR362567	AR362567 Sequence
12	281	37.6	2704	6	AR364854	AR364854 Sequence
13	280.8	37.6	397	10	MUSTCRAAE	M87852 Mus musculu
14	280.8	37.6	432	10	MMTCRB7	X02784 Mouse mRNA
15	280.8	37.6	486	10	MMU31879	U31879 Mus musculu
16	280.8	37.6	748	10	MMTCRAV1	X02857 Mouse T-cel
17	280.8	37.6	110000	10	AE008685_2	Continuation (3 of
18	280.8	37.6	122176	10	AC003997	AC003997 Mouse BAC
19	278.8	37.3	666	10	MUSTCRBC	L37870 Mus musculu
20	278.8	37.3	685	10	MUSTCRBF	L37872 Mus musculu
21	278.8	37.3	804	10	MMU83243	U83243 Mus musculu
22	278.8	37.3	954	6	A97417	A97417 Sequence 7
23	278.8	37.3	954	10	MMAJ158	AJ000158 Mus muscu
24	278.6	37.3	366	6	AR122581	AR122581 Sequence
25	278.6	37.3	366	6	AR211754	AR211754 Sequence
26	278.6	37.3	366	6	AR401594	AR401594 Sequence
27	278.6	37.3	747	6	AR122583	AR122583 Sequence
28	278.6	37.3	747	6	AR211756	AR211756 Sequence
29	278.6	37.3	747	6	AR401596	AR401596 Sequence
30	278.6	37.3	753	6	AR122582	AR122582 Sequence
31	278.6	37.3	753	6	AR211755	AR211755 Sequence
32	278.6	37.3	753	6	AR401595	AR401595 Sequence
33	278.2	37.2	319	10	MUSTCRBH	L37869 Mus musculu
34	278.2	37.2	338	10	MUSTCRBG	L37873 Mus musculu
35	278.2	37.2	510	10	MUSTCBVC4	M15617 Mouse germl
36	278.2	37.2	621	6	AR362560	AR362560 Sequence
37	278.2	37.2	621	6	AR364850	AR364850 Sequence
38	278.2	37.2	621	10	MUSTCBXZY	M19404 Mouse rearr
39	278.2	37.2	688	10	MUSTCBXT	M13669 Mouse T-cel
40	278.2	37.2	250611	10	MMAE000663	AE000663 Mus muscu
41	278.2	37.2	261600	10	AC125228	AC125228 Mus muscu
42	277.2	37.1	405	10	MUSIG63A9A	M26417 Mus musculu
43	277	37.1	652	10	MUSTCRBD	L37871 Mus musculu
44	276.4	37.0	496	10	MUSTCR8B	M98568 Mouse T cel
45	275.4	36.9	486	10	MMU31877	U31877 Mus musculu

ALIGNMENTS

RESULT 1
AR220110
LOCUS AR220110 747 bp DNA linear PAT 26-SEP-2002
DEFINITION Sequence 24 from patent US 6423538.
ACCESSION AR220110
VERSION AR220110.1 GI:23324541
KEYWORDS .
SOURCE Unknown.
ORGANISM Unknown.
Unclassified.
REFERENCE 1 (bases 1 to 747)
AUTHORS Wittrup,K.D., Kranz,D.M., Keike,M. and Boder,E.T.
TITLE Yeast cell surface display of proteins and uses thereof
JOURNAL Patent: US 6423538-A 24 23-JUL-2002;
FEATURES Location/Qualifiers
source 1. .747
/organism="unknown"
/mol_type="genomic DNA"
ORIGIN

Query Match 100.0%; Score 747; DB 6; Length 747;
Best Local Similarity 100.0%; Pred. No. 1.9e-202;
Matches 747; Conservative 0; Mismatches 0; Indels 0; Gaps 0;

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Qy          1 GACGTCGCAGTCACCCAAAGCCCAAGAAACAAGGTGGCAGTAACAGGAGGAAAGGTGACA 60
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Db          1 GACGTCGCAGTCACCCAAAGCCCAAGAAACAAGGTGGCAGTAACAGGAGGAAAGGTGACA 60

Qy         61 TTGAGCTGTAATCAGACTAATAACCACAACAACATGTACTGGTATCGGCAGGACACGGGG 120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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Qy        121 CATGGGCTGAGGCTGATCCATTATTCATATGGTGCTGGCAGCACTGAGAAAGGAGATATC 180
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Qy        181 CCTGATGGATACAAGGCCTCCAGACCAAGCCAAGAGAACTTCTCCCTCATTCTGGAGTTG 240
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Qy        241 GCTACCCCCTCTCAGACATCAGTGTACTTCTGTGCCAGCGGTGGGGGGGGCACCTTGTAC 300
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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'Institute of Medical Im-
the Humboldt University,
arité, Medical Dept of the
y

ıe variable region of the
ly CB41 and a detailed
by peptide mutations led
model of the antigen-an-
shows two hydrophobic
a salt bridge (L:Arg50)
rgy. In order to verify the
d and expressed a scFv-
roperties (scFv41, KD =
ıportant residues by site
H:Tyr32Ala scFv lacking
no binding activity. The
scFv with out the salt
KD. These results seem to
ertheless, first crystallo-
L3-loop conformation in

essig, S., Hausdorf, G.,
sner, H., Giessmann, E.,
d Schomburg, D. (1993)

mutant of antibody D1.3
nced binding to hen egg

F.P. Schwarz[a,b], R.J. Pol-
search in Biotechnology,
of Chemical Engineering,
'01, USA.

lation by phage display of
which bind with improved
Interestingly, no residues
rface were altered in the
side chains can influence
such a way as to improve
is reported to bind HEL

hange upon HEL binding
l the mutant M3, using
find that ΔΔH and ΔΔCp
type. ΔΔG will be deter-
ormed by surface plasmon
quench titrations. Prelim-
lpic stabilization achieved
ıvourable configurational
ons to binding free energy
hydrophobic effect will be
hod of Freire et al. [2]
of mutant antibodies with
e, help guide the in vitro
oviding structural insights
hanced binding.

[1] Hawkins et al. (1993) J. Mol. Biol. 234, 958.
[2] Freire et al. (1995) Proteins 21, 83.

Isolation and characterization of human single chain Fv (scFv) against botulinum neurotoxin type A.

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Botullnum neurotoxins, which cause the flaacid paralysis associated with the disease botulism, are proteins composed of two polypeptide chains. The light chain possesses the enzymatic activity and the heavy chain is responsible for binding to neuronal membranes. The carboxy-terminal half of the heavy chain (H_C) mediates neurospecific binding and the amino-terminal half of the heavy chain (H_N) assists in internalization to the toxin. To produce human antibodies capable of toxin neutralization, a human immune scFv phage antibody library was generated using peripheral blood lymphocytes of an individual immunized with botulinum penta-valent toxoid (ABCDE). The V_H and V_L genes were amplified by PCR and spliced together to create an scFv gene repertoire which was cloned into pCANTAB 5E (Pharmacia) to create a library of 7.7×10^5 members. Hybridisation of the unselected library using V_λ or V_κ light chain specific primers demonstrated 66% V_κ light chain genes and 33% V_λ light chain genes. The library was selected on botulinum toxin type A (BTA) immobilized on polystyrene and after 4 rounds of panning, 84 out of 92 clones bound the toxin. Nucleotide sequencing revealed that 26 different scFv antibodies have been isolated. Native scFv were expressed to determine their specificity by ELISA on BTA, recombinant BTA translocation domain and C-fragment domain, 15 of these bound BTA, but not H_C or H_N. Four scFv bound both BTA and H_N. Seven scFv bound both BTA and H_C. Binding studies of scFv against the H_C, which is believed to play a key role in neutralization of the toxin, will be presented.

Antibodies as modulators of protein activities — implications for intravenous therapy.

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We have examined the modulating effects of non-immune human IgG on the activities of biospecific molecules like antibodies and enzymes.

Non-immune human IgG was found to inhibit the binding of antigen by specific antibodies of both human and rabbit origin. Human immuno-globulins were also able to modify the composition of preformed antigen-antibody complexes. Furthermore, the presence of non-immune human IgG was found to affect the activity of enzymes (yeast glucose-6-phosphate dehydrogenase and human placental alkaline phosphatase). We have also observed some odd interactions of immunoglobulins with antibodies used as affinity ligands.

None of the observed effects could be explained only as a result of activities of specific antibodies in the non-immune IgG preparations. Taken together, our results suggest that immunoglobulins may interact with each other and with other proteins not only as antibodies against antigens, but also through interactions which are distinct from antigen-binding.

A network of such 'non-immunological' interactions would be of great importance in providing suitable conditions for physiological protein activities and may, at least in part, explain the beneficial effects of intravenous therapy in autoimmune conditions. It is also easy to conceive a regulatory function of immunoglobulins similar to the allosteric regulation of, for instance, enzymatic activities through this kind of interactions.

Yeast surface display system for antibody engineering.

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Progress in antibody engineering has been largely stimulated by methodological advances. Phage display technology is a powerful and polular means to generate new antibodies and to mature existing antibodies for improved affinity or specificity through successive rounds of mutagenesis and selection by 'panning'. This technique requires expression of the antibody library in *E. coli*, a host organism which exhibits a strong expression bias against many heterologous proteins. Conversely, the protein processing and secretory machinery of the yeast *Saccharomyces cerevisiae* bears striking homology to that of mammalian cells, while the organism remains easily manipulable through molecular genetics techniques. Thus, yeast is an ideal choice for the expression of libraries of antibodies or other mammalian proteins for the purpose of directed evolution.

A surface display system for the *in vitro* expression and selection of peptide and protein libraries on yeast has been developed. A nine residue peptide epitope (HA) has been fused to the binding subunit of a yeast cell wall protein (AGA2), followed by the 4-4-20 anti-fluorescein single-chain F_v. Selection was performed by fluorescence activated cell sorting (FACS). Single-pass and double-pass enrichment factors have been determined by FACS performed on mixtures of cells with and without the displayed fusion. This system presents the potential for the in vitro affinity maturation of antibodies as well as the directed evolution of other proteins and peptides, with the advantages of (i) a double-label FACS selection scheme allowing finer affinity discrimination than panning; (ii) as many as 10^4 copies of the displayed sequence per cell, eliminating stochastic variations in the selection; and (iii) library expression in yeast, with an altered ot potentially improved expression bias which could yield clones which would be deleted from a library expressed in *E. coli*.

A bacterial surface-expression system using OmpA fusion-proteins.[4]

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